Bioinformatics Approaches to Identify the Comorbidity Complexities of *SARS-CoV-2* Infection with Crohn's Disease

Yue Li[1] and Xiao Han[2]

[1]*Department of Emergency and Critical Care Medicine, Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai, China*

[2]*Department of Cardiology, Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai, China*

ABSTRACT

Objective: To analyse potential molecular mechanisms and identify potential therapeutic regimens and drugs to treat severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*) and Crohn's disease (CD) through bioinformatics and systems biology.

Study Design: Bioinformatics Study.

Place and Duration of the Study: Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai, China, from May to December 2022.

Methodology: The common differentially expressed genes (DEGs) between CD and *SARS-CoV-2* infection were identified using two RNA-seq datasets (GSE147507, GSE153974) extracted from Gene Expression Omnibus (GEO). Subsequently, functional enrichment, pathway analysis, and candidate drug analysis were performed using these DEGs.

Results: In total, 44 DEGs were identified as common between CD and *SARS-CoV-2* infection. A protein-protein interaction (PPI) network was constructed, hub genes were identified, and critical modules were determined by means of bioinformatics and combinatorial statistical approaches. Functional and pathway analyses conducted under ontological conditions showed a common association between CD and infection with *SARS-CoV-2*. The common DEGs were then used to identify coregulatory networks of interactions between transcriptional factors and genes, between proteins and medicines, and between DEGs and miRNAs.

Conclusion: Top 10 hub genes including *IL6, CXCL1, CSF2, CXCL2, CXCL5, MMP3, PTGS2, CXCL3, SELE,* and *LCN2* were identified, which may function as potential candidate targets for *SARS-CoV-2* infection. Additionally, the identification of certain promising treatment drugs for patients with *SARS-CoV-2* infection and CD was also made.

Key Words: *SARS-CoV-2, COVID-19, Drug molecule, Hub gene, Protein-protein interaction (PPI), Gene ontology, Crohn's disease, Differentially expressed genes.*

How to cite this article: Li Y, Han X. Bioinformatics Approaches to Identify the Comorbidity Complexities of *SARS-CoV-2* Infection with Crohn's Disease. *J Coll Physicians Surg Pak* 2023; **33(10)**:1093-1099.

INTRODUCTION

Crohn's disease (CD) is an inflammatory disorder that primarily affects the gastrointestinal (GI) tract and is hallmarked by chronic granulomatous inflammation.[1] The severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*) is responsible for the coronavirus disease 2019 (COVID-19) epidemic.[2] In their clinical presentations, the CD and *SARS-CoV-2* infection share many symptoms, including abdominal pain, diarrhoea, and pneumonia.

Correspondence to: Dr. Xiao Han, Department of Cardiology, Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai, China
E-mail: y12091209@126.com

Received: December 25, 2022; Revised: August 12, 2023; Accepted: August 26, 2023
DOI: https://doi.org/10.29271/jcpsp.2023.10.1093

The previous studies have shown that absorptive enterocytes obtained from inflamed ileal tissues express higher levels of ACE2 compared to those from uninflamed tissues which suggest that ACE2 may possess anti-inflammatory activities and protect against injury.[3] In patients with CD, elevated ACE2 expression levels in tissues inhibit inflammatory signals by activating the Ang-(1-7)/Mas pathway. Additionally, elevated ACE2 expression levels are associated with the amino acid transporter B0AT-1, which facilitated the absorption of tryptophan to modulate the microbiota-gut-brain axis. Hence, a crucial question that arises is whether patients with CD (those expressing intestinal ACE2) are at a greater risk of contracting *SARS-CoV-2* infection and the relevant cytokine release syndrome. The elevated levels of ACE2 that are seen in CD patients are downregulated upon infection with *SARS-CoV-2*, breaking the innate immunity, disrupting the transportation of amino acids, and impairing the homeostatic balance of intestinal microecology, which further aggravates the digestive tract symptoms.[4,5] The *SARS-CoV-2* infection could cause or exacerbate intestinal inflammation due to its systemic symptoms. So, it is imperative to understand how *SARS-CoV-2* infection

affects patients with CD and develop candidate drugs that can alleviate the associated symptoms and complications. As of yet, no bioinformatics study has looked at the impact of *SARS-CoV-2* at the molecular level in patients with pre-existing CD.

Disease-specific datasets GSE147507 (*SARS-CoV-2*) and GSE153974 (CD) were compared from the Gene Expression Omnibus (GEO) database to bioinformatics analysis.

METHODOLOGY

The present study utilised microarray and RNA-seq datasets obtained from the GEO database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/) to explore the common genetic pathways between *SARS-CoV-2* and CD. The research was conducted in Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai, China. The authors screened the protein sequences using the following criteria to obtain a high-quality dataset: (1) genome variation profiling by high-throughput sequencing; (2) case-control studies of *Homo sapiens*; (3) series type; (4) expression profiling by array; (5) comparable conditions for experimental and untreated controls; (6) analysis of gene expression profiling; (7) availability of completely raw and processed microarray data; and (8) the appropriate positioning for RNA extraction.

The *SARS-CoV-2* dataset with GEO accession ID GSE147507 was obtained and included high-throughput sequencing using the Illumina HiSeq 500 platform for RNA-seq extractions from lung biopsies of 23 patients (including lung samples derived from a single male COVID19 deceased patient (age: 74 years) with *SARS-CoV-2* infection; and 55 healthy controls (including uninfected human lung biopsies were derived from one male (age 72 years) and one female (age 60 years) in the USA. Next, the dataset chosen for analysis, with the accession ID GSE153974, comprised of 57 samples of CD and 17 samples of healthy control individuals in UK. These samples consisted of human ileal biopsies that were preserved in RNA, later and subsequently sequenced using the high-throughput Illumina NextSeq 500 (*Homo sapiens*) sequencing system. Figure 1(a) shows the summary of the datasets.

DESEq2 R package was applied to identify DEGs for the GSE147507 and GSE153974 datasets, with the criteria set as |log fold change| ≥1.0 and false discovery rate (FDR) <0.05. Subsequently, the identification of the common differentially expressed genes (DEGs) was facilitated by the utilisation of Jvenn (http://jvenn.toulouse.inra.fr/app/example.html), an online tool for Venn diagram analysis.[6] The biological mechanisms and Reactome signalling pathways of the common differentially expressed genes (DEGs) were analysed, and the top-ranked functional items and pathways were identified using a standardised metric of p-value <0.05.

In order to demonstrate the interconnectedness of *SARS-CoV-2* and CD at both a structural and functional level, the differentially expressed genes (DEGs) were uploaded to the STRING database (http://string-db.org/), the goal of which is to evaluate protein-protein interactions (PPI) and integrate the results. The PPI network of overlapping DEGs was generated based on a low confidence score of 0.15. Additionally, the Cytoscape program (v.3.8.0), a commonly employed bioinformatics tool for the visualisation of biological networks and integration of associated data, was utilised in the study to further visualise and investigate the PPI network.

The 10 prominent hub genes in the protein-protein interaction (PPI) network were identified through the application of the Cytohubba MCC technique. Subsequently, their rankings were visually represented using a colour gradient ranging from red to yellow. Additionally, the shortest paths between hub genes were determined by using the proximate neighbourhood ranking features of Cytohubba.

Through the utilisation of the NetworkAnalyst online tool (http://www.networkanalyst.ca/), an exploration was conducted to identify transcriptional factors (TFs) that bind to the shared differentially expressed genes (DEGs). To identify miRNAs that bind to transcripts of target genes and hence, adversely affect protein production by interfering with the messenger RNA (mRNA) stability and translational efficiency, the NetworkAnalyst online tool was employed to conduct an analysis of miRNA-target gene interactions sourced from MiRTarBase. Then, the researchers screened for high-degree miRNAs and determined the biochemical processes and characteristics associated with each miRNA.

Enrichr and the Drug Signatures Database (DSigDB) were employed to investigate the molecular composition of drugs by analysing shared differentially expressed genes (DEGs) between *SARS-CoV-2* infection and CD. DSigDB is an updated gene set repository for use in gene set enrichment analysis (GSEA) that relates compounds/drugs and their respective target genes.

By utilising the DisGeNET online tool (http://www.disgenet.org/), a comprehensive analysis was conducted to identify multiple chronic conditions associated with the common differentially expressed genes (DEGs).

RESULTS

In the dataset of *SARS-CoV-2* infection, a total of 1781 differentially expressed genes (DEGs) were identified, comprising 391 downregulated and 1390 upregulated genes. Likewise, 253 DEGs were identified in the CD dataset, among which 4 were downregulated, whereas 249 were upregulated. All the DEGs were identified using the criteria of p-value <0.05 and an absolute log fold value (|logFC|) ≥1. A p-value <0.05 suggested that there is a 5% risk of making a false decision to choose a DEG as per the cutoff threshold. LogFC is a log ratio between gene expression levels and is used for calculating differences in expression levels in two distinct conditions such as control *vs*. case. Figure 1b shows the volcano plots and heatmap of the identified DEGs in the *SARS-CoV-2* infection (Figure 1(bi)) and CD (Figure 1(bii)) datasets. By employing Jvenn, a total of 44 differentially expressed genes (DEGs) that were shared between the two datasets were identified (Figure 1c), indicating that several genes are similar under *SARS-CoV-2* infection and CD conditions, and that the pathogenesis may be closely related.

a

Disease name	GEO accession	GEO platform	Total DEGs count	Up regulated DEG count	Down regulated DEGs count
SARS-CoV-2	GSE147507	GPL18573	1781	1390	391
CD	GSE153974	GPL18573	253	249	4

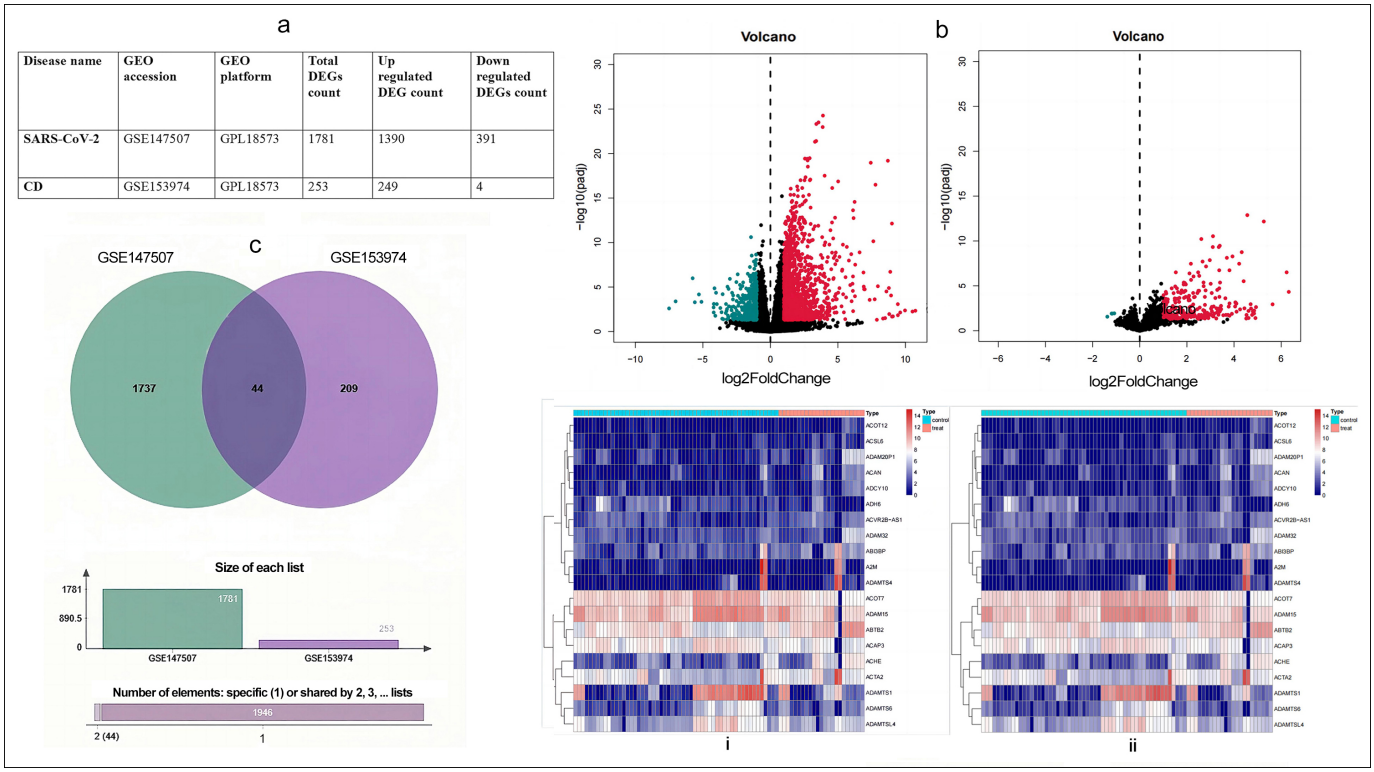


Figure 1: (a) Summary of the geographical characteristics and quantitative analytical measurements of the selected data sets. (b) Discovery of DEGs between *SARS-CoV-2* infection and CD. The red dots in the volcano plots in (i) and (ii) represent significant DEGs for *SARS-CoV-2* infection and CD, respectively. The criteria for detecting DEGs are |logFC| ≥1 and adjusted p-value <0.05. Based on log2 fold changes, the heatmaps show the relationships between the common DEGs of different conditions. DEG, differentially-expressed genes; CD, Crohn's disease. (c) Venn diagram showing the number of common DEGs between *SARS-CoV-2* infection (GSE147507) and CD (GSE153974) datasets.

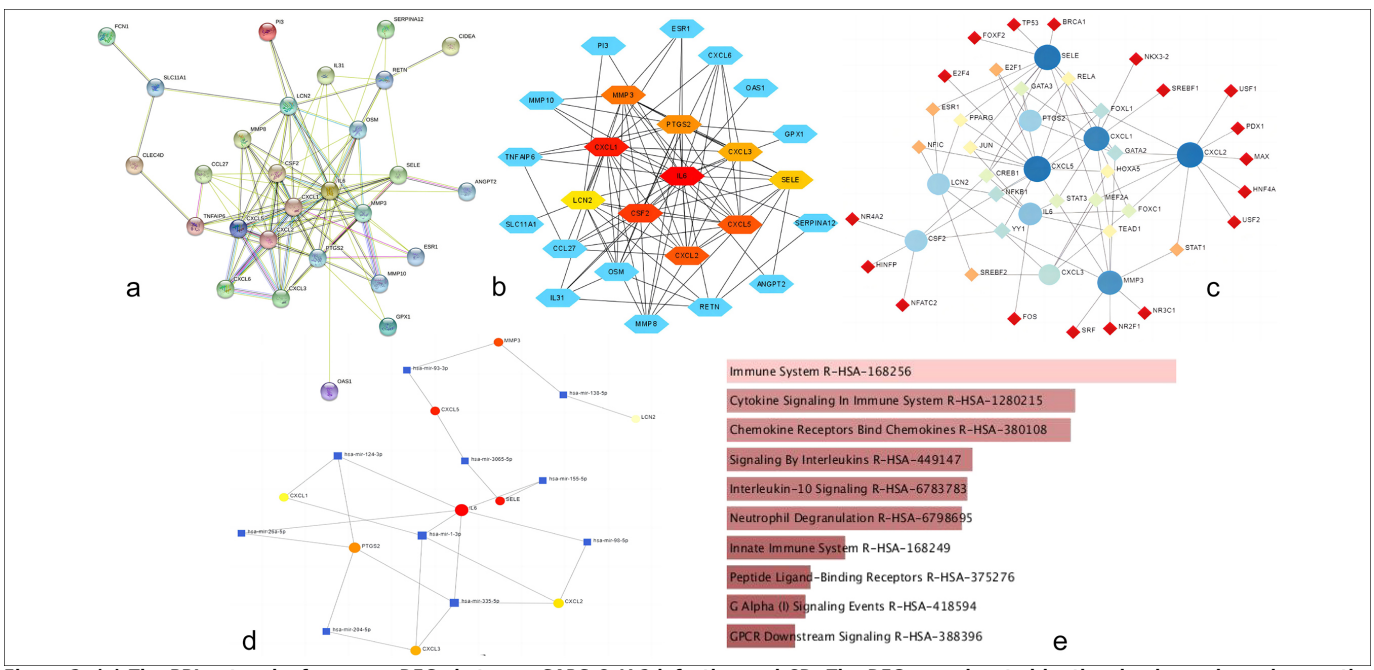


Figure 2: (a) The PPI network of common DEGs between *SARS-CoV-2* infection and CD. The DEGs are denoted by the circular nodes, whereas the corresponding interactions are denoted by the edges. The PPI network consists of 44 nodes and 100 edges. DEGs, differentially expressed genes; CD, Crohn's disease; PPI, protein-protein interaction. (b) Detection of hub genes. Genes that serve as hubs in the PPI network were found utilising the Cytohubba Cytoscape plugin. The top-ranked 10 hub genes and the molecules they interact with are displayed in red, orange, or yellow. There are 25 nodes in the submodule network, connected by 96 edges. (c) Interactions between the DEGs and TFs. Circles indicate the DEG, while squares represent TFs. 47 nodes and 83 edges were found in the network. TF, transcription factor; DEGs, differentially expressed genes. (d) Interactions between the DEGs and microRNAs (miRNAs). Circles represent miRNAs, and squares represent the genes that interact with them. The network has 19 nodes and 25 edges. DEGs, differentially expressed genes. (e) Reactome 2022 Pathway enrichment analysis of the common DEGs between *SARS-CoV-2* infection and CD is presented as bar graphs.

Table I: Medicine candidates in treating *SARS-CoV-2* infection and Crohn's disease (CD).

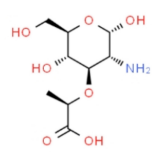
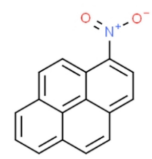
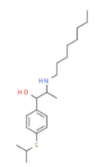
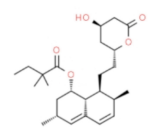
Name	p-values	Chemical formula	Structure
Peptidoglycan CTD 00006490	6.90E-11	$C_9H_{17}NO_7$	
1-Nitropyrene CTD 00001569	7.24E-11	$C_{16}H_9NO_2$	
Suloctidil PC3 UP	1.50E-09	$C_{20}H_{35}NOS$	
Simvastatin and Niacin BOSS	1.98E-07	$C_{25}H_{38}O_5$	

Figure 2(a) illustrates the PPI network shared between *SARS CoV-2* infection and CD. The PPI network comprised of 44 nodes and 100 edges. Furthermore, through the use of the Cytoscape plugin Cytohubba, the researchers discovered the hub proteins of the PPI network as depicted in Figure 2(b). Overall, 10 DEGs (22.73%) were considered to be the most influential genes, including *IL6, CXCL1, CSF2, CXCL2, CXCL5, MMP3, PTGS2, CXCL3, SELE,* and *LCN2*. These hub genes could help identify drugs for diseases associated with these comorbidities. In order to enhance comprehension of their proximity and connectivity, a submodule network consisting of 25 nodes and 96 edges was constructed utilising the Cytohubba plugin (Figure 2(b)). The analyses of DEG-TF and DEG-miRNA interaction were conducted with the help of NetworkAnalyst (http://www.networkanalyst.ca/) to determine the transcription and post-transcription regulatory biomolecules controlling the DEGs. The interplay between modulatory TFs and DEGs based on the JASPAR database is shown in Figure 2(c). The *TFs YY1, GATA2, FOXL1*, and *NFKB1* were found to be the most common DEGs between *SARS-CoV-2* infection and CD. The interplay between DEGs and miRNA regulators based on the miRTarBase v8.0 database is depicted in Figure 2d. The most common miRNAs between *SARS-CoV-2* infection and CD were found to be hsa-mir-1-3p, hsa-mir-335-5p, hsa-mir-124-3p, hsa-mir-155-5p, and hsa-mir-3065-5p. Additionally, 47 TFs and 19 miRNA regulatory signatures were associated with multiple DEGs, demonstrating a strong association between them.

Gene enrichment analysis can determine the chromosome position and disease-related biological response of a gene. Pathway annotation analysis was conducted to investigate whether the DEGs are involved in biological processes or metabolic pathways. The authors examined the global databases, namely Reactome to determine the pathways impacted by the common DEGs between *SARS-CoV-2* infection and CD. For a more detailed explanation, bar graphs based on the pathway enrichment analysis are shown in Figure 2e. In this enrichment analysis, the DEGs are listed in increasing order based on p-value, with a p-value of 0.05 being considered significant.

Researchers identified 10 potential drug molecules for *SARS CoV-2* infection and CD using Enrichr based on DSigDB. As per the p-value, Table I lists the top four candidate compounds, which may be effective for targeting the common DEGs.

DISCUSSION

In this study, 44 DEGs shared similar expression patterns in CD and *SARS-CoV-2* infection. Within the biological process, the cytokine-mediated signalling pathway (16 genes) and cellular response to cytokine stimulus (13 genes) ranked the highest. Cytokines are released by immune and non-immune cells and cause inflammation and tissue damage in the gut of patients with CD.[7] It is known that IBD-related inflammation occurs due to faulty production and activity of anti-inflammatory cytokines. Inflammation can be managed with anti-cytokine therapy that targets the immune system to restore intestinal barrier function. In patients with *SARS-CoV-2* infection, a cytokine storm prevents further virus transmission and induces secondary tissue damage by secreting large amounts of inflammatory mediators. The growing evidence indicates that *SARS-CoV-2* infection severity is associated with a cytokine storm, which is also a critical cause of death.

Specific granules (8 genes) and tertiary granules (7 genes) were found to be the top two GO pathways in the cellular component category. There is evidence that zymogen-granule membrane glycoprotein 2 (GP2) is one of the most frequent targets of autoantibodies in CD. Furthermore, the cytotoxic granule proteins produced by eosinophils are closely associated with CD.[8] In *SARS-CoV-2* infection, platelet granule release is uncoupled from integrin activation, suggesting that GPIIb/IIIa can be therapeutically targeted.[9] In terms of biological processes, cytokine activity (11 genes) and CXCR chemokine receptor binding (5 genes) were among the most prominent GO terms in the present analysis. The inflammation that led to tissue damage in CD is triggered by cytokines and the severity of *SARS-CoV-2* infection was related to the cytokine storm. Moreover, treatment with antibodies against chemokine ligand (CXCL) 13 (the ligand of CXCR5) reduced the severity of colitis.[10] The IL-8-CXCR-1/-2 axis targeting suppressed the activation of neutrophils, NETosis, degranulation, and IL-8 production, which led to a reduced number of *SARS-CoV-2* spike protein-induced, ACE2-mediated microthromboses in the lungs.

Ten hub proteins, namely *IL6, CXCL1, CSF2, CXCL2, CXCL5, MMP3, PTGS2, CXCL3, SELE,* and *LCN2*, were found to be involved in the two diseases based on the PPI network.

IL-6 is a pleiotropic cytokine involved in immune system regulation among many other extensive functions. As a powerful pro-inflammatory cytokine, IL-6 is an important component involved in the response of the immune system to pathogens and acute stress. Cancer, inflammation, and autoimmunity are pathologically related to IL-6 pathways. In *SARS-CoV-2* infection, exploratory studies have suggested that a significant increase in IL-6 levels is associated with adverse outcomes. Hence, the inhibition of IL-6 may be one of the most promising therapeutic targets for patients with *SARS-CoV-2* infection with dysregulated host responses.[11] Meanwhile, *CXCL1, CXCL2,* and *CXCL5* are the proinflammatory cytokines responsible for CD inflammatory responses. A correlation between MMP-3 and MMP-9 concentrations and CD severity suggests that they may be useful in evaluating CD severity, while MMP3 is currently being investigated for its potential as a biomarker as well as a drug target in *SARS CoV-2* infection. LCN2 is a non-invasive biomarker that correlates with intestinal inflammation. Moreover, there is potential for neutrophil-related LCN2 to serve as a new biomarker to anticipate the severity of *SARS-CoV-2* infections, and it may also be used for new treatment targets. Thus, these hub genes can potentially function as disease biomarkers or as novel therapeutic targets, if the biological knowledge gained on *SARS-CoV-2* infection can be verified.

Subsequently, the researchers proceeded to establish associations among DEGs, TFs, and miRNAs. The different types of digestive system disorders appeared to be associated with the TFs identified (*YY1, GATA2, FOXL1, NFKB1, MEF2A, CREB1, FOXC1, STAT3,* and *GATA3*). Moreover, several of the identified miRNAs are implicated in lung cancer (e.g., hsa-mir-1-3p, hsa-mir-138-5p, hsa-mir-26a-5p), hepatocellular carcinoma (e.g., hsa-mir-26a-5p, hsa-mir-124-3p, hsa-mir-1-3p) and osteosarcoma (e.g., hsa-mir-335-5p, hsa-mir-26a-5p).[12-14] Therefore, these molecules are worth further testing as new therapeutic targets for treating the symptoms of *SARS-CoV-2* infection. In essence, these transcription factors and microRNAs target proteins to influence disease progression. For instance, there has been a research to demonstrate that hsa-mir-335-5p and hsa-mir-98-5p target IL6 and hsa-mir-1-3p and hsa-mir-98-5p target CXCL1.[15] Additionally, four miRNAs have been predicted (hsa-mir-1-3p, hsa-mir-335-5p, hsa-mir-124-3p, and hsa-mir-155-5p) to be associated with different CD-related genes.

The association between DEGs and different diseases was predicted using gene–disease analysis. Different illnesses have been linked to *SARS-CoV-2* infection, such as mouth neoplasms, pancreatic neoplasms, colorectal neoplasms, bladder neoplasms, kidney neoplasms, esophageal neoplasms, and stomach neoplasms. A study has also shown a close correlation between *SARS-CoV-2* and cancer risk, mortality, and severe illnesses. Tumour cells can be stimulated to switch metabolisms by using *SARS-CoV-2* to initiate metabolic modes with higher production efficiency, which would promote tumour progression and facilitate *SARS-CoV-2* replication.[16] An alternative study suggested that tumour suppressor genes are inhibited by *SARS-CoV-2*, possibly promoting cancer onset. Additionally, a correlation between *SARS-CoV-2* and autoimmune diseases was also discovered. Several studies indicated that patients with systemic autoimmune disease have a higher chance of developing *SARS-CoV-2* infection.[17]

The curative radical therapy of *SARS-CoV-2* is being explored in greater detail in this study. In the present investigation, the researchers anticipated the identification of four potential pharmaceutical compounds for the treatment of *SARS-CoV-2*. Peptidoglycan was identified, which acts against enveloped viruses by disrupting virion integrity. A study has shown that enterovirus inactivation can be affected by lipopolysaccharide or peptidoglycan of bacterial origin.[18] 1-NITROPYRENE can cause apoptosis and pyroptosis in cells. A form or forms of cytochrome P450 are selectively induced by 1-NITROPYRENE to facilitate the metabolism of the substance. 1-NITROPYRENE induces macrophage synthesis of proinflammatory cytokines including, interleukin 1 (IL-1), IL-6, and TNF, which exhibit proinflammatory responses.[19] Therefore, there is a possibility that 1-NITROPYRENE may enhance virus inactivation and lead to an immune response to help patients fight *SARS-CoV-2*. An antithrombotic drug, suloctidil, as a potential *SARS-CoV-2* therapeutic agent, has been reported as an antifungal agent, especially against *Candida albicans.*[20] This drug may play a significant role in patients infected with *SARS-CoV-2* complicated with fungal infections and pulmonary embolism.

Furthermore, simvastatin and niacin were approved by the FDA to lower lipids and may enhance patients' recovery outcome in *SARS-CoV-2* infection. The nucleocapsid protein (NP) of *SARS-CoV-2* strongly activates human endothelial cells *via* the MAPK and TLR2/NF-κB pathways, while simvastatin significantly inhibits endothelial activation induced by NP, thus suppressing virus cell entry and inflammatory cytokine production.[21,22]

To date, there has been no comprehensive examination of the molecular-level effects of *SARS-CoV-2* on individuals with pre-existing CD in both local and international bioinformatics research. The findings of this study lend support to future pharmaceutical studies on the candidate drugs to treat patients with CD and *SARS-CoV-2* infection. However, notwithstanding our diligent endeavours, this study does possess certain limitations. Insufficient sample size and the different selection criteria of the datasets may hinder the identification of common DEGs in disease studies, thereby potentially limiting the comprehensive capture of all necessary disease-associated genes. Therefore, an additional investigation is necessary to comprehensively

evaluate the biological significance of the potential target candidates identified in this study. It is worth mentioning that the above results were obtained by applying bioinformatics analyses without experiments or clinical trials. Therefore, further experiments are necessary to verify the safety and efficacy of the potential medicines against CD and *SARS-CoV-2* infection.

CONCLUSION

The top 10 hub genes were identified, namely *IL6*, *CXCL1, CSF2, CXCL2, CXCL5, MMP3, PTGS2, CXCL3, SELE*, and *LCN2* between CD and *SARS-CoV-2* infection. Moreover, the analysis identified the TFs and miRNA that play an important role in *SARS-CoV-2* infection, thus highlighting potential risk factors. Five potential drugs were also predicted: Peptidoglycan (a component of bacterial cell walls), 1-NITROPYRENE, suloctidil, simvastatin, and niacin, which may lead to new therapeutic targets and the development of *SARS-CoV-2* infection vaccines.

ACKNOWLEDGEMENT:
This work was supported by the Scientific Research Foundation of Shanghai University of Medicine and Health Sciences (SSF-21-16-001) and Shanghai University of Medicine and Health Sciences Clinical Research Centre for Metabolic Vascular Diseases Project (20MC2020004).

ETHICAL APPROVAL:
The study was approved by the Ethics Committee of Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai, China (Approval No. XHEC-C-2022-12).

PATIENTS' CONSENT:
Written informed consents were obtained from patients to publish the data.

COMPETING INTEREST:
The authors declare no potential competing interest with respect to the research, authorship, or publication of this article.

AUTHORS' CONTRIBUTION:
YL: Manuscript writing and data analysis.
XH: Subject design and data review.
All authors have approved the final version of the manuscript to be published.

REFERENCES

1. Rosmaninho A, Sanches M, Salgado M, Alves R, Selores M. Vulvoperineal Crohn's disease responsive to metronidazole. *An Bras Dermatol* 2013; **88(6 Suppl 1)**:71-4. doi: 10.1590/ abd1806-4841.20132243.
2. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of *SARS-CoV-2* and COVID-19. *Nat Rev Microbiol* 2021; **19(3)**:141-54. doi: 10.1038/ s41579-020-00459-7.
3. Yan S, Ye P, Aleem MT, Chen X, Xie N, Zhang Y. Mesenchymal stem cells overexpressing ACE2 Favorably ameliorate LPS-induced inflammatory injury in mammary epithelial cells. *Front Immunol* 2021; **12**:796744. doi: 10.3389/fimmu.2021. 796744.
4. Yisireyili M, Uchida Y, Yamamoto K, Nakayama T, Cheng XW, Matsushita T, *et al*. Angiotensin receptor blocker irbesartan reduces stress-induced intestinal inflammation *via* AT1a signaling and ACE2-dependent mechanism in mice. *Brain Behav Immun* 2018; **69**:167-79. doi: 10.1016/j. bbi.2017. 11.010.
5. Yu Z, Yang Z, Wang Y, Zhou F, Li S, Li C, *et al.* Recent advance of ACE2 and microbiota dysfunction in COVID-19 pathogenesis. *Heliyon* 2021; **7(7)**:e07548. doi: 10.1016/j. heliyon.2021.e07548.
6. Jia A, Xu L, Wang Y. Venn diagrams in bioinformatics. *Brief Bioinform* 2021; **22(5)**:bbab108. doi:10.1093/bib/bbab108.
7. MacDonald TT, Biancheri P, Sarra M, Monteleone G. What's the next best cytokine target in IBD? *Inflamm Bowel Dis* 2012; **18(11)**:2180-9. doi: 10.1002/ibd.22967.
8. Loktionov A. Eosinophils in the gastrointestinal tract and their role in the pathogenesis of major colorectal disorders. *World J Gastroenterol* 2019; **25(27)**:3503-26. doi: 10.3748/ wjg.v25. i27.3503.
9. Weiss LJ, Drayss M, Manukjan G, Zeitlhöefler MJ, Kleiss J, Weigel ML, *et al*. Uncoupling of platelet granule release and integrin activation suggests GPIIb/IIIa as therapeutic target in COVID-19. *Blood Adv* 2022. doi: 10.1182/blood advances. 2022008666.
10. Nagy-Szakal D, Mir SAV, Harris RA, Dowd SE, Yamada T, Lacorazza HD, *et al*. Loss of n-6 fatty acid induced pediatric obesity protects against acute murine colitis. *FASEB J* 2015; **29(8)**:3151-9. doi: 10.1096/fj.14-267690.
11. Ramasamy S, Subbian S. Critical determinants of cytokine storm and Type I interferon response in COVID-19 pathogenesis. *Clin Microbiol Rev* 2021; **34(3)**:e00299-20. doi: 10. 1128/CMR.00299-20.
12. Chen Y, Chen Q, Zou J, Zhang Y, Bi Z. Construction and analysis of a ceRNA-ceRNA network reveals two potential prognostic modules regulated by hsa-miR-335-5p in osteosarcoma. *Int J Mol Med* 2018; **42(3):**1237-46. doi: 10.3892/ ijmm.2018.3709.
13. Ma T, Liu A, Xu D, Zhang T. Mechanisms underlying the promotion of osteosarcoma cell proliferation and invasion by lncRNA PBB12. *Oncol Rep* 2020; **43(2)**:736-46. doi:10. 3892/ or.2019.7451.
14. Luo T, Yi X, Si W. Identification of miRNA and genes involving in osteosarcoma by comprehensive analysis of microRNA and copy number variation data. *Oncol Lett* 2017; **14(5)**:5427-33. doi: 10.3892/ol.2017.6845.
15. Sun Y, Cai D, Hu W, Fang T. Identifying hub genes and miRNAs in Crohn's disease by bioinformatics analysis. *Front Genet* 2022; **13**:950136. doi: 10.3389/fgene.2022. 950136.
16. Li YS, Ren HC, Cao JH. Correlation of *SARS-CoV-2* to cancer: Carcinogenic or anticancer? (Review). *Int J Oncol* 2022; **60(4)**:42. doi: 10.3892/ijo.2022.5332.

17. Brito-Zerón P, Sisó-Almirall A, Flores-Chavez A, Retamozo S, Ramos-Casals M. *SARS-CoV-2* infection in patients with systemic autoimmune diseases. *Clin Exp Rheumatol* 2021; **39(3)**:676-87. doi: 10.55563/clinexprheumatol/lekp1y.

18. Waldman P, Meseguer A, Lucas F, Moulin L, Wurtzer S. Interaction of human enteric viruses with microbial compounds: Implication for virus persistence and disinfection treatments. *Environ Sci Technol* 2017; **51(23)**: 13633-40. doi: 10.1021/ acs.est.7b03875.

19. Tsai PK, Chen SP, Huang-Liu R, Chen CJ, Chen WY, Ng YY, *et al*. Proinflammatory responses of 1-Nitropyrene against RAW 264.7 Macrophages through Akt phosphorylation and NF-κB pathways. *Toxics* 2021; **9(11)**:276. doi: 10.3390/toxics911 0276.

20. Zeng B, Li J, Wang Y, Chen P, Wang X, Cui J, *et al*. In vitro and *in vivo* effects of suloctidil on growth and biofilm formation of the opportunistic fungus *Candida albicans*. *Oncotarget* 2017; **8(41)**:69972-82. doi:10.18632/onco target.19542.

21. Qian Y, Lei T, Patel PS, Lee CH, Monaghan-Nichols P, Xin HB, *et al*. Direct activation of endothelial cells by *SARS-CoV-2* nucleocapsid protein is blocked by simvastatin. *J Virol* 2021; **95(23)**:e0139621. doi: 10.1128/JVI.01396-21.

22. Teixeira L, Temerozo JR, Pereira-Dutra FS, Ferreira AC, Mattos M, Gonçalves BS, *et al*. Simvastatin downregulates the *SARS-CoV-2*-induced inflammatory response and impairs viral infection through disruption of lipid rafts. *Front Immunol* 2022; **13**: 820131. doi: 10.3389/fimmu.2022.820131.